ISOLATION AND IDENTIFICATION OF LACTIC ACID BACTERIA ON BOILER CHICKEN

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Abstract: This research aimed to isolate lactic acid bacteria from broiler chicken. The primary material was a chicken boiler aged 30 days. The identification of LAB was carried out based on isolation and identification of morphological, phenotypic, and biochemical characteristics. Thirteen LAB isolates were obtained, and 5 selected LAB isolates were identified based on growth levels, pH, and lactic acid levels. Five selected LAB isolates were identified as *Lactobacillus* genus, which has Gram-positive, negative catalase, non-motility, hetero-fermentative, rod shapes, rod-colony shapes, and cream colony colour.
Keywords: isolation; identification; lactic acid bacteria; meat broiler

Abstrak: Penelitian ini bertujuan untuk mengisolasi bakteri asam laktat dari ayam broiler. Bahan utamanya adalah ayam boiler berumur 30 hari. Identifikasi BAL dilakukan berdasarkan isolasi dan identifikasi karakteristik morfologi, fenotipik, dan biokimia. Tiga belas isolat BAL diperoleh dan 5 isolat BAL terpilih diidentifikasi berdasarkan tingkat pertumbuhan, pH, dan kadar asam laktat. Lima isolat BAL terpilih diidentifikasi sebagai genus *Lactobacillus* yang memiliki Gram-positif, katalase negatif, non-motilitas, hetero-fermentatif, bentuk batang, bentuk koloni batang, dan warna koloni krem.
Kata kunci: isolasi; identifikasi; bakteri asam laktat; daging ayam broiler

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Introduction

Lactic acid bacteria (LAB) are members of Gram-positive, non-spore, spherical (cocci), or rod-shaped (bacilli), which are produce lactic acid as the main product of carbohydrate and catalase-negative fermentation. Members of the LAB genus were classified into *Aerococcus*, *Carnobacterium*, *Enterococcus*, *Tetrageonococcus*, *Vagococcus*, *Lactobacillus*, *Pediococcus*, *Leuconostoc*,

Oenococcus, *Weissella*, *Lactococcus*, and *Streptococcus* (Wright & Axelsson, 2012).

Lactic acid bacteria could grow on the plant (Kuda et al., 2016), fish (Sarika et al., 2018), chicken digestive (Blajman et al., 2015), and chicken neck skin (Sakaridis et al., 2014). The high nutritional value and carbohydrate on the meat could be good nutrition for growing bacteria. The carbohydrate on the meat was stored as glycogen (Alghannam et al., 2018). Lactic acid bacteria have been extensively used for preservation technique such as fermentation over the last decades. The lactic acid produced by LAB was a useful compound on food preservation as they could maintain the acidic condition of fermentation product, and they could be able to retain inhibitory properties against pathogenic and spoilage which cause food spoilage and food poisoning. Lactic acid bacteria could be used to prevent coliform growth (Sirilun et al., 2017), *B. cereus, E. coli, S. typhimurium,* and, *Listeria monocytogenes* (Gómez et al., 2016). Lactic acid bacteria can produce an antibacterial compound due to produce lactic acid to reduce the pH (Ren et al., 2018). Lactic acid is the main product of food fermentation, while the lactic acid also produces acetic acid, propionic acid, malic acid, and another acid. Lactic acid bacteria could protect against the pathogenic bacteria and increases the food shelf life (Hernández-Aquino et al., 2019). The research of isolation from chicken meat which could be used for chicken meat fermentation is rarely reported. To be used for fermentation on chicken meat, it necessary to select the lactic acid bacteria which produce the highest level of lactic acid, the lowest pH, and the fastest of lactic acid bacterial growth. Meanwhile, to determine the genus of lactic acid bacteria needs identification.

This research aimed to obtain the lactic acid bacterial isolate (LAB) which had the highest lactic acid concentration from isolated boiler chicken and identification of LAB cell isolates.

Materials and Method

Research Materials

This research was carried out by a set of glass tools, analytical scales (Ohaus), UV-Vis spectrophotometer, pH meter (Hanna), oven (Memmert), laminar airflow (Telstar Bio-IIA/P), microscope.

The primary materials in this research were: boiler chicken aged 30 days, while other materials such as de Mann Rogosa Sharpe (MRS) (Merck), de Mann Rogosa Sharpe Agar (MRSA) (Merck) with pH 6.2, alcohol 70 %, aquadest, hydrogen peroxide (H_2O_2) 3 % and Gram dyes (violet crystal, iodine, acetone, safranin), and NaOH.

Research Method

Lactic Acid Bacteria Isolation (LAB)

Probiotic candidate bacterial were isolated from boiler chicken aged 35 days which was taken one hour after slaughtering. The samples were taken from the chest by cutting with a sterile knife, to avoid contaminating bacteria. Boiler chicken (10g) was mashed and added with 90ml of 0.85 % physiological NaCl solution. From the suspension, serials dilutions were made for each sample by taking 1 ml into 9 ml of physiological NaCl and making it up to 10^{-6}. The dilution (1 ml) was spread on a petri dish containing MRS agar plates which added with $CaCO_3$ (1 %) (b/v) and then incubated at 37 ºC for 48 h. Lactic acid bacteria colonies were growing and formed a clear zone around the colony. It could happen due to the lactic acid produced by bacteria could dissolve the calcium carbonate ($CaCO_3$) (Chen et al., 2017).

The growth colonies with distinct morphologies (such as colour, shape, and size) were randomly selected (Liu et al., 2012). The established colonies were grown on the MRS agar and then incubated in a test tube with an upside-down position at 37 ºC for 48 h.

The selected colonies grew apart were move into purification. The purification was carried out by re-isolation method with streak technique to ensure its purity.

The initial screening was obtained by selecting the isolates then tested for pH value according to their morphology (shapes, colours, and sizes). Further screening was carried out by selecting isolates that have low pH then were tested for growth and lactic acid levels (Nurhayati, 2008).

The pH was measured using a pH meter (Hanna) that had been calibrated with a standard solution (pH 4 and pH 7). The pH electrode was inserted into the sample (10 ml), and all the treatments were repeated in every measurement.

Test Growth and LAB Isolates

10 % of LAB isolate was inoculated into a Hungate tube containing MRS Broth. Lactic acid bacteria isolates were incubated at 37 ºC. To analyze the LAB isolate growth, the optical density (OD) was observed at 600nm of wavelength every hour for 13 hours. The repetitions were carried out three times (Astuti, 2010).

Lactic Acid on LAB Isolates Test

The lactic acid test using the method described by Baker and Sumerson (Hawk, 1976). The lactic acid was isolated from free-protein samples (protein was precipitated using TCA 20 %) by $CuSO_3$ and $CaOH_2$ compounds. H_2SO_4 heated the lactic acid to converted into acetaldehyde. The density of acetaldehyde was measured using a spectrophotometer with 560 nm wavelength. The lactic acid level could be calculated from the standard lactic acid equation and the

absorbance that has been analyzed before. 1 mg of lithium lactate was dissolved into 5 ml aquadest to made the main soluble.

Identification of LAB Isolates Cellular from Boiler Chicken

The identified isolates were identified as phenotypic and biochemical identifications. The phenotypic identification test consists of a Gram stain, colony morphology, motility, catalase, and fermentation type test. While the biochemical identification test consists of a growth test at 10 ºC, 37 ºC and 45 ºC, at pH 4.4 and 9.6 and on sodium chloride levels of 6.6 % and 18 %. (Wright & Axelsson, 2012). All the treatments were repeated in every measurement.

Staining Gram Test

This test is done by the method of (Cappuccino & Sherman, 2014). The first LAB isolate to be observed were regenerated by growing on the MRS agar media and incubated at 37 ºC for 2 days. The colonies were tested for Gram stain. The staining test using four types of solutions, such as base (crystal violet), Mordant (lugol), dyestuffs (alcohol), and other stains (counterstains), namely safranin solutions. The first stage to stained the Gram was cleaned the glass object with a sterile cotton swab then gave the labels on it. The bacterial culture on slant agar was taken using a sterile loop and transferred into the centre of the glass object, then adding sterile distilled water. The dried preparation was fixed on a bunsen burner than in each preparation was dripped with crystal violet, lugol, alcohol (95 %), and safranin (10-30 s), and rest for 1 min then rinsed with distilled water and dried using a tissue. The preparation objects were dripped with immersion oil before observed the cell shape using a microscope (magnification 100x40). The gram-positive cell was indicated with purple colour meanwhile gram-negative cells with red colour. All the treatments were repeated in every measurement.

Motility Test

The bacterial motility test was described by (Cappuccino & Sherman, 2014). Lactic acid bacteria isolate taken with a loop needle, inserted into the MRS broth containing 0.5 % agar were incubated for 1-2 days at 37 ºC. The motility is determined based on the least growth bacteria on the media, and it could be indicated positive if the LAB isolates growth spreads on soft media. All treatments were made in duplicate and repeated.

Catalase Test

The catalase test was carried out according to the method (Cappuccino & Sherman, 2014). Catalase test is done by dropping one to two H_2O_2 (3 %) on one loop of the bacterial isolate. Positive test reaction showed when the bubbles are formed. All the treatments were made in duplicate and repeated.

Fermentation Type Test

This test was described by the method of (Cappuccino & Sherman, 2014). Fermentation type testing was conducted by gas production test, which growing the isolated culture in MRS liquid media. Lactic acid gas was caught by reverse the Durham tube. Furthermore, the LAB was incubated for 2-3 days at 30 ºC. Lactic acid bacteria have two types of fermentation. The first is homo-fermentative when there were not show bubbles and the second is hetero-fermentative when there were show bubbles in the Durham tube.

Test Growth on Temperature (10 ºC, 37 ºC, and 45 ºC)

The test depended on Cappuccino & Sherman, (2014) method. This test was conducted by growing isolate cultures in MRS liquid media at 10 ºC, 37 ºC, and 45 ºC for 24 hours and analyze through visualized at the colony isolates sediment. All the treatments were made in duplicate and repeated.

Test Growth on pH (4.4 and 9.6)

The growth of LAB isolate on pH 4.4 and 9.6 was described by(Cappuccino & Sherman, 2014). This test is done by observing the LAB isolate on MRS liquid media at pH 4.4 and 9.6. Then it was incubated for 24 hours. The visual analysis was done for the LAB isolates sediment in the tube reaction. All the treatments were made in duplicate and repeated.

Test Growth on Sodium Chloride Level

Growth test on sodium chloride levels 6.5 % and 18 % according to the method of (Cappuccino & Sherman, 2014). Isolate culture on MRS liquid media added with sodium chloride level 6.6 % and 18 %, and it was incubated for 24 hours then the visual observations were done on the LAB isolate sediment. All the treatments were made in duplicate and repeated.

Data analysis

The isolation and identification of LAB probiotics from broiler chicken were analyzed descriptively. The characteristics of selected LAB isolates was carried out by comparing the typical characteristics (Wright & Axelsson, 2012).

Result and Discussion

Isolation on Probiotic Lactic Acid Bacterial from Boiler Chicken

Lactic acid bacteria isolated from boiler chicken was identified by the pour plate method at 10^{-2} and 10^{-3} on MRS agar medium. Twenty-one pure isolate colonies were obtained from 70 candidate LAB isolate colonies based on their morphology (shape, size, and colour) (Liu et al., 2012). Table 1 was shown for 21 selected LAB isolate that screened by measuring pH value. The result shows that the pH values ranged from 3.39 to 6.08.

Table 1. pH Value of Selected LAB

Number	LAB Isolate Code	pH	Used/Unused
1	BR 12	3.39	Used
2	BR 6	3.59	Used
3	BR 4	3.60	Used
4	BR 11	3.62	Used
5	BR 10	3.65	Used
6	BR 17	3.76	Used
7	BR 7	3.81	Used
8	BR 19	4.30	Used
9	BR 5	4.32	Used
10	BR 1	4.47	Used
11	BR 8	4.55	Used
12	BR 3	4.60	Used
13	BR 2	4.61	Used
14	BR 20	5.38	Unused
15	BR 18	5.53	Unused
16	BR 9	5.73	Unused
17	BR 16	5.74	Unused
18	BR 15	6.05	Unused
19	BR 13	6.06	Unused
20	BR 14	6.08	Unused
21	BR 21	6.08	Unused

The pH value from the screening result was obtained in 13 isolates has ranged from 3.39- 4.61. Thirteen isolates were grown on MRS Broth media to measure the growth, lactic acid levels, and pH values. The isolate growth test was shown in Figure 1.

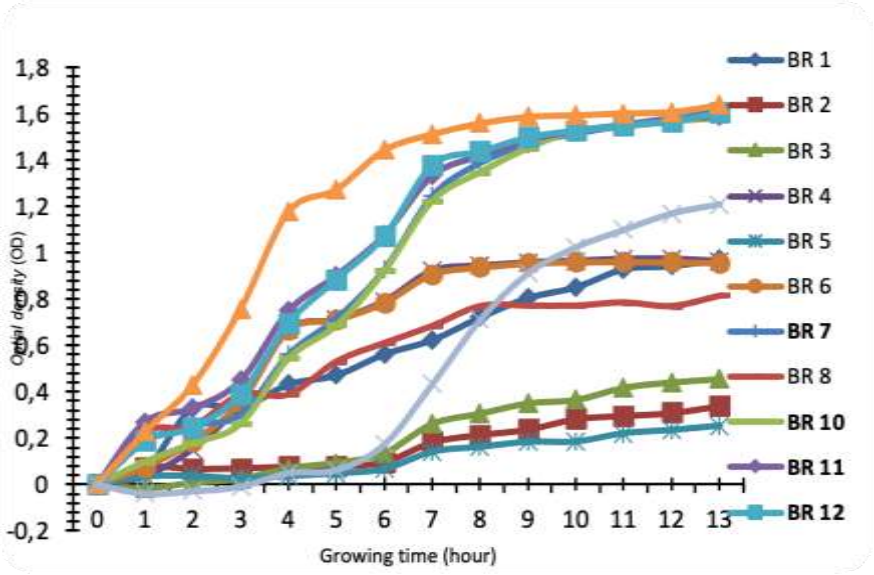

Figure 1. Graph of 13 selected isolate from boiler chicken on liquid media.

Selected isolates growth was measured at an incubation time of 0-12 hours. Figure 1 shows that 5 isolates (BR11, BR10, BR12, BR7, and BR17) had the highest growth rate. The Optical Density (OD) values of 5 selected BAL isolates were mention in Table 2. Its shows that 5 selected BAL isolates have different OD value. The result confirmed by Hidayati (2010) that the further growth of LAB isolates due to differences in the ability of LAB to utilized the carbohydrate source.

Table 2. The OD Value of 5 Selected LAB Isolates on MRS Broth Media

Time (hour)	LAB Isolate Code					Average
	BR 7	BR 10	BR 11	BR 12	BR 17	
0	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00^{a} ± 0.00
1	0.18 ± 0.02	0.09 ± 0.01	0.27 ± 0.01	0.19 ± 0.00	0.23 ± 0.00	0.19^{b} ± 0.00
2	0.26 ± 0.02	0.18 ± 0.04	0.33 ± 0.00	0.25 ± 0.01	0.43 ± 0.01	0.29^{c} ± 0.02
3	0.30 ± 0.02	0.26 ± 0.05	0.45 ± 0.00	0.39 ± 0.01	0.76 ± 0.01	0.43^{d} ± 0.02
4	0.56 ± 0.03	0.55 ± 0.05	0.75 ± 0.01	0.69 ± 0.02	1.18 ± 0.00	0.75^{e} ± 0.06
5	0.72 ± 0.03	0.69 ± 0.04	0.90 ± 0.02	0.89 ± 0.02	1.27 ± 0.01	0.89^{f} ± 0.06
6	0.93 ± 0.02	0.91 ± 0.03	1.08 ± 0.01	1.07 ± 0.02	1.44 ± 0.01	1.09^{g} ± 0.05
7	1.24 ± 0.00	1.22 ± 0.00	1.33 ± 0.01	1.38 ± 0.02	1.51 ± 0.01	1.34^{h} ± 0.03
8	1.39 ± 0.01	1.35 ± 0.02	1.42 ± 0.01	1.44 ± 0.01	1.56 ± 0.01	1.43^{i} ± 0.02
9	1.47 ± 0.01	1.45 ± 0.01	1.49 ± 0.01	1.49 ± 0.00	1.59 ± 0.01	1.49^{j} ± 0.01
10	1.52 ± 0.01	1.53 ± 0.01	1.53 ± 0.01	1.53 ± 0.01	1.59 ± 0.01	1.54^{k} ± 0.01
11	1.55 ± 0.01	1.54 ± 0.01	1.55 ± 0.01	1.55 ± 0.00	1.60 ± 0.01	1.56^{l} ± 0.00
12	1.57 ± 0.00	1.57 ± 0.01	1.58 ± 0.01	1.57 ± 0.01	1.61 ± 0.01	1.58^{l} ± 0.01
13	1.63 ± 0.02	1.59 ± 0.02	1.59 ± 0.00	1.60 ± 0.01	1.64 ± 0.00	1.61^{m} ± 0.04
Average	0.95^{b} ± 0.09	0.92^{a} ± 0.09	1.02^{c} ± 0.09	1.00^{d} ± 0.09	1.17^{d} ± 0.09	

^{abc}Different superscripts on the same line showed a very significant effect ($P < 0.05$)
^{xy}Different superscripts in the same column showed a significant effect($P < 0.05$)

The growth curve of the 5 highest selected LAB isolates was illustrated in Table 1. The lag phase occurs for less than one hour. Meanwhile, the log phase ends at 10 hours. It found faster than the result reported by Wu et al., (2009) which states that in 0-3 hour of fermentation in the lag phase of L. casei Zhang was occurred, while the log phase ends at 10th hour of fermentation. The lag phase of *L*. fermentum IFO 3956, which isolated from cheese indicated at 0-4 hours, meanwhile the log phase occurred from 4-12 hours of fermentation (El-Ghaish et al., 2010). The representative of lactic acid and pH value on 5 selected LAB isolates are shown in Table 3.

Table 3. Lactic Acid Level and pH Value on LAB Isolates from Boiler Chicken on Liquid Media

Number	Isolates Code	pH Value	Lactic Acid Level (mg/mL)
1	BR 12	3.39	12.09
2	BR 11	3.62	10.41

Number	Isolates Code	pH Value	Lactic Acid Level (mg/mL)
3	BR 10	3.65	8.17
4	BR 17	3.76	11.32
5	BR 7	3.81	7.47

Thirteen selected isolates produce lactic acid ranges from 3.45-12.26 mg/ml. The result shows that there is a correlation between the number of lactic acids in boiler chicken and digestive chicken, the results still in the ranges of 3.12-19.21 mg/ml (Nurhayati, 2008). The highest level of lactic acid shows respectively BR 12, BR 17, BR 11, BR 10, and BR 7. Glucose as a substrate being used by LAB to produce lactic acid as the main fermentation product (Wright & Axelsson, 2012).

The lowest and the highest result of pH value was found in the range of 3.39 and 4.61. The pH value result of boiler chicken confirmed still on ranges number 3.77-4.68 of digestive chicken (Nurhayati, 2008). Thirteen selected isolates were selected based on the lowest pH value. The lowest pH value from 5 selected isolates shows respectively isolate BR 12, BR 11, BR 10, BR 17 dan BR 7.

Identification of Lactic Acid Bacteria Isolates from Boiler Chicken

The phenotypic identification of 5 selected isolates was observed by gram stain, shape, motility test, catalase test, cell structure, colony colour, and fermentation type. The phenotypic identification of boiler chicken LAB can be seen in Table 4.

The result shows that 5 LAB isolates were classified as Gram-positive, catalase-negative, non-motile, hetero-fermentative, rod shape, rod colony shape, and colony colour were cream. This indicated that all isolates were obtained as LAB. The result is related to Monika et al., (2017), that LAB is a Gram-positive, catalase-negative and non-motile.

Table 4. Phenotypic Identification of LAB Isolates from Boiler Chicken

Isolates Code	Gram Marked	Catalase	Motility	CO_2 Production	Cell Shape	Colony Shape	Colony Color
BR 7	+	-	-	+	Rod	Cocci	Cream
BR 10	+	-	-	+	Rod	Cocci	Cream
BR 11	+	-	-	+	Rod	Cocci	Cream
BR 12	+	-	-	+	Rod	Cocci	Cream
BR 17	+	-	-	+	Rod	Cocci	Cream

The staining method aimed to differentiate the bacteria based on the cell wall structure. Gram-positive have a thick peptidoglycan layer (90 % of the cell wall), and it will indicate with blue to purplish colour meanwhile at the end of the staining method, the colour of LAB will indicate a purple colour (Ray & Bhunia, 2013).

Figure 2 shows that the colonies of LAB isolate from the chicken boiler were classified in a rod. The result could be indicated that the LAB isolates from the genus *Carnobacterium* (Wright & Axelsson, 2012).

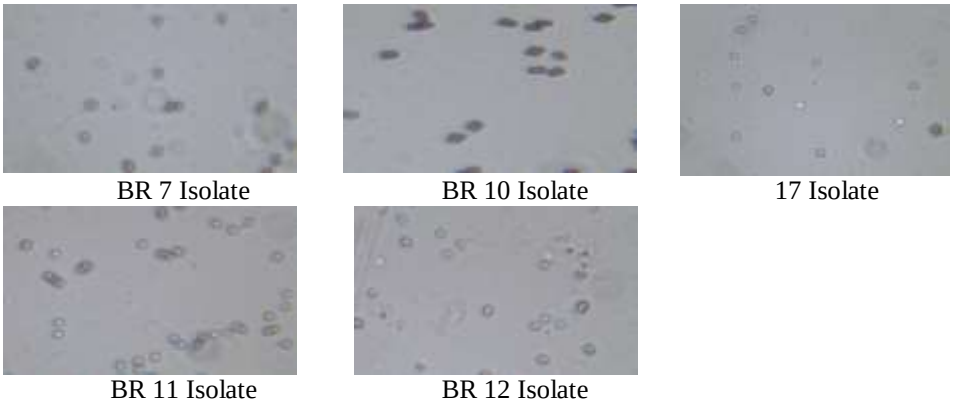

Figure 2. LAB Isolates Morphology Test Result (400x Magnification)

The growth of bacteria indicated the negative motility result just in the point of inoculation. Meanwhile, the positive result was shown that the colony spread out of the effectiveness of inoculation. The LAB is a non-motile was declared by (Mokoena, 2017).

Catalase test was performed to detect the existence of catalase enzyme in bacteria. The catalase enzyme could neutralize the bactericidal effect of hydrogen peroxide. Catalase could accelerate the breakdown of hydrogen peroxide into water and oxygen ($2H_2O_2$ + catalase $\leftrightarrow$ $2H_2O + O_2$). The absence of formation of the bubble indicates that the catalase-positive of isolate (there was no catalase enzyme hydrolyzes the hydrogen peroxide). Based on the result, the LAB isolates show as catalase-negative. Mokoena (2017) said that the LAB is catalase-negative.

The fermentation type test was used to classify LAB into homo-fermentative and hetero-fermentative groups. Based on the main fermentation, lactic acid was classified as a homo-fermentative group, while the hetero-fermentative group needs not only lactic acid, but also ethanol, CO_2., and other acids (Mokoena, 2017). The main fermentable sugar is lactose, thus lactic acid bacteria that produce β- galactosidase can hydrolyze lactose into fermentable sugars of glucose and galactose. The CO_2 carried out the fermentation type test. The glucose in enzyme bacteria could break down the compound, and the bacteria that ferment glucose would produce gas (CO_2). The carbon dioxide would dissolve in the water, while hydrogen gas did not dissolve in the water so that the hydrogen would be trapped in the Durham tube and formed as bubbles at the top of the tube. Based on the result, LAB isolates could identify as hetero-fermentative bacteria because of the gas detected trapped in the Durham tube.

Leuconostoc, *Oenococcus*, and *Weissella* genus are classified as hetero-fermentative, while *Lactobacillus* genus could classify as hetero-fermentative and hetero-fermentative (Wright & Axelsson, 2012). The result shows that LAB isolates have a rod-shaped and hetero-fermentative it might state as *Lactobacillus* genus.

Biochemical identification of LAB isolates includes the ability to grow at various growth temperatures (10 ºC, 37 ºC, 45 ºC), pH values (4.4 and 9.6), and salinity (6.5 % dan 18 %) (Monika et al., 2017). The identification can be seen in Table 5.

Table 5. LAB Isolate Biochemical Identification Test Result

Isolates Code	Growth of Isolates						
	Temperature (°C)			pH Value		(NaCl) (%)	
	10	37	45	4.4	9.6	6.5	18
BR 7	GS	+	+	+	-	+	-
BR 10	GS	+	+	+	-	+	-
BR 11	GS	+	+	+	-	+	-
BR 12	GS	+	+	+	-	+	-
BR 17	GS	+	+	+	-	+	-

(+) Growing ; (-) Not Growing: (GS) Growing Slowly

Table 5 could be explained that at 10 ºC of temperature, the LAB isolates grow slowly, while at 45 ºC the LAB isolate could grow. Lactic acid bacteria were classified as mesophilic bacterial growth at temperature ranges 10-45 °C. The optimal temperature for the growing lactic acid bacteria is 30-45 ° C (Ardhana & Fleet, 2003). LAB isolated from chicken gizzard could grow at temperatures of 10 ºC, 15 °C, 40 °C, and 45 °C is the *Lactobacillus* genus, namely *Lb. Fermentum, Lb. Plantarum, Lb. Brevis dan Lb. Casei ssp.pseudoplantarum* (Idoui, 2014).

The ability growth test of LAB isolates was grown at pH 4.4 and pH 9.6. Based on the result, LAB isolates could grow and positively in pH 4.4, and it was one of the base classifications to classify the LAB genus. Table 5 shows that 5 selected LAB isolates can grow in pH 4.4. Gram-positive bacteria could grow in pH ranges 4.0-8.5; however, some species could grow at a lower pH (Ray & Bhunia, 2013). *Leuconostocs* and *streptococcus* usually could grow in lower pH around 4.0 to 4.5 and some *Lactobacilli* and *Pediococci* around pH 3.5.

Lactic acid bacteria could be isolated from meat fermentation. It could obtain isolates 11, 14, and 24 which could grow at a pH of 4.4 (Mulaw et al., 2019) meanwhile, Yunilas et al., (2014) report that LAB could also be isolated from beef and its shown that the isolates unable to grow at pH 9. The ability growth test for LAB isolates at pH 9.6 showed that all isolates have negative results.

The salinity result shows that LAB isolates could grow by 6.5 %, and did not increase by 18 % salinity. The LAB isolated from kefir obtained SK-1 and

SK-4, which could grow in 6.5 % salinity (Ismail et al., 2018). Lactic acid bacteria from the genus *Lactobacillus plantarum* strain JCM 1149 were able to grow in 6.5 salinity (Mulaw et al., 2019). Lactic acid bacteria isolate strain SFE-7(33) and P12A(25) which isolated from shrimp intestines were able to grow in 5-10 % salinity but could not grow 18 % salinity (Romadhon et al., 2012).

Based on phenotypic and biochemical identification shows that BR 7, BR 10, BR 11, BR 12, and BR 17 isolates were classified as Gram-positive, catalase-negative, non-motile, rod shapes, hetero-fermentative, grow slowly at 10 ºC, grow fast at 37ºC and 45 ºC, pH 4.4, and 6.5 % salinity, did not succeed in pH 9.6 and 18 % salinity.

Based on the morphological identification, bacterial cell growth, and phenotypic characteristics, it might conclude that all isolates belong to the Lactobacillus genus. Lactic acid bacteria with the Lactobacillus genus has a rod shape, could be homo-fermentative and hetero-fermentative, could grow at temperatures of 10 ºC, 37 ºC, and 45 ºC, 6.5 % salinity and pH 4.4 (Wright & Axelsson, 2012). *Lactobacillus* and *Carnobacterium* genus are LAB which has a rod shape. The *Carnobacterium* genus only has homo-fermentative and did not grow at 45 ºC, 6.5 % and 18 % salinity, and pH 9.6.

Five selected LAB isolates from boiler chicken analyzed by isolation and identification were considered as the same genus, namely *Lactobacillus*. However, the differences have been selected for different shapes, sizes and colours. This result due to the growth of *Lactobacillus* bacteria grows faster than *Streptococcus*. It was confirmed by Hasan et al., (2016) who state that the growth of L. bulgaricus faster than L. rhamnosus. The slowest growth was *S. Thermophilus* Syah et al., (2017) also confirmed that LAB isolates from fresh dangke (traditional cheese) obtained 30 isolates, mostly were rods (28 isolates) and cocci (2 isolates). The same result was also reported by (Arief et al., 2015) who isolated LAB from beef found that 11 isolates identified as *Lactobacillus plantarum*, 6 isolates as *Lactobacillus acidophilus*, 2 isolates as *Pediococcus pentosaceus* and 1 isolate as *Enterococcus faecium*.

Conclusions

Five lactic acid bacteria isolates have been selected from 13 LAB isolates boiler chicken and were identified as *Lactobacillus*. Based on highest growth, lower pH, and higher lactic acid level, the 5 selected LAB isolates were identified as Gram-positive, negative catalase, non-motility, hetero-fermentative, rod shapes, rod-colony shapes, and cream colony colour. Five selected LAB isolates grow slowly in 10 ºC, grow faster in ranges 37 ºC dan 45 ºC, pH 4.4 and 6.5 % of salinity meanwhile, the isolates did not succeed in pH 9.6 and 18 % of salinity.

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